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(54) Simultaneous multiple assays and compounds and compositions useful therein.

(57) The present invention is concerned with coordinated compounds useful in simultaneous multiple assays for organic species such as steroids, proteins, peptides, carbohydrates or drugs. The coordinated compounds are prepared by labelling an individual organic species with a radioisotope through a chelating agent to form a coordinated compound.

The present invention is also concerned with compositions comprising two or more of the coordinated compounds, compositions comprising one or more of the coordinated compounds and an analyte labelled with I-125, and a simultaneous multiple assay wherein one or more of the coordinated compounds is are employed.

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SIMULTANEOUS MULTIPLE ASSAYS AND COMPOUNDS AND COMPOSITIONS USEFUL THEREIN.

The present invention is concerned with simultaneous multiple assays and compounds and compositions useful therein. The simultaneous multiple assays may be carried out for organic species such as steroids, proteins, peptides, carbohydrates or drugs.

Radioimmunoassay is an analytical technique that resulted from the work of Berson and Yalow. In radioimmunoassay, radiolabelled exogenous antigen competes with unlabelled endogenous antigen for binding sites on an antibody or specific binding proteins, e.g. intrinsic factor, specific for the antigen.

The percentage of bound radiolabelled antigen decreases as a function of the increasing concentration of unlabelled antigen in the test sample. Separation of the bound and free radiolabelled antigen is necessary in order to determine the quantity of unlabelled antigen. This can be accomplished by insolubilization of the antigen-antibody complexes either by chemical means, e.g., polyethylene glycol precipitation, or by the addition of a second antibody directed toward the immunoglobulin present in the original antiserum, or by a combination of these two methods. The quantity of unlabelled antigen in an unknown sample is then determined by comparing the radioactivity of the precipitate, after centrifugation, with values established using known standards in the same assay system.

In one aspect this invention is concerned with simultaneous measurement of two or more organic species in the same tube wherein the material to be assayed is radiolabelled. It is also concerned with the preparation of the labelled organic species employing chelating agents.

There is a continuing search for cheaper and quicker analytical procedures. One way to accomplish this is to have an assay whereby two or more organic species can be

assayed simultaneously in the same solution.

An example is in U.S. Patent No. 4,146,602 issued on March 27, 1979 which discloses a simultaneous assay of Folate and Vitamin B_{12}.

Co^{57} is incorporated in Vitamin B_{12} which is rather uncomplicated since Vitamin B_{12} is a cobalt containing compound.

The problem was how to incorporate Co^{57} into non-cobalt containing organic species.

Certain uses of chelating agents are well known; however, there is no known use of chelating agents to prepare radiolabelled organic species useful in simultaneous assays.

A paper by Yeh et al. at pages 327-336 of J. Radioanal Chem., 53, (1979) describes the preparation of an assay of indium chelates. A chapter in the American Chemical Society publication Advances In Chemistry Series, No. 198 Modification of Proteins by Meares et al. at pages 369-387 discusses chelate tagged proteins and polypeptides using cobalt to prepare radiopharmaceuticals.

Egan et al at pages 611-613 of a paper entitled "^{57}Co: A Volume Mark for the TRIPLE-ISOTOPE, Double-Antibody Radioimmune Assay" in Immunochemistry, 1977, Vol. 14, discusses using a chelating agent (EDTA) with cobalt; but to prevent adsorption of cobalt to serum proteins.

We have now found that by employing chelating agents it is possible to label different organic species with different nuclides to provide a method for simultaneous assays on multiple organic species in a single tube.

Instruments are already being used to read radioactivity in a simultaneous assay (Vitamin B_{12} and folate). Therefore, this invention will not require any new techniques or instrumentations.

According to one aspect of the present invention there is provided a coordinated compound of the formula:

metal isotope--chelator--organic species.

Further aspects of the present invention concern a composition useful in a simultaneous assay comprising two or more coordinated compounds according to the invention, a composition useful in a simultaneous assay comprising one or more coordinated compounds according to the invention and an analyte labelled with I-125, and a simultaneous multiple assay using one or more coordinated compounds according to the invention.

The coordinated compound of the present invention can conveniently be incorporated in a kit for use in simultaneous multiple assays of organic species.

By the use of this invention one can place a metal isotope on any suitable organic species, e.g. analyte, to assay for said organic species.

Another aspect is the metal isotope labelling of purified antibodies to said analyte(s) to construct an immunoradiometric assay (IRMA).

The essence of the invention is the introduction of radionuclides into organic species by way of organic species-bound chelating moieties and the subsequent use of the radio-labelled organic species in radioassays.

The chelators may be of a variety of materials satisfying the following criteria:

1) they must be capable of forming covalent linkages with the organic species of interest;

2) once attached to the organic species, they must retain their ability to form coordination complexes with +2 and +3 metal radionuclides; and

3) the formed complexes of organic species, chelator, and metal radionuclides must retain all or part of the binding specificity or antigenicity of the native organic species.

Radiolabelled organic species containing individually distinguishable radionuclides may be combined in a variety of configurations such that one or more organic species may be measured simultaneously by radioassay. Organic species labelled in this described manner could also be combined with organic species labelled by alternate means to provide simultaneous radioassays.

The choice of radionuclides to be utilized for labelling is governed by the following practical considerations.

1) they must have a sufficiently long half-life to enable them to be used over a practical period of time (eg. several months);

2) they must be available in sufficiently high specific activity to provide an adequate signal amplification; and

3) they must possess a distinguishable emmission spectrum when used in combination with one or more other radionuclides.

The method of assaying comprises employing a coordinated compound of the general formula:

metal isotope--chelator--organic species

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Examples of analytes which can be employed include any organic species which can react with a chelating agent. In general, they are steroids such as estrogens, progesterone, digoxin, cortisol, 17-hydroxyprogesterone and the like; proteins, such as human chorionic gonadotropin (HCG), luteinizing hormone (LH), follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), alpha-fetoprotein, trypsin, hepatitis associated antigen, carcinoembryonic antigen and the like; peptides, such as ACTH, endorphins, angiotensin, insulin and the like;

carbohydrates, such as pneumococcal polysaccharides and the like; drugs, such as cocaine, tetrahydrocannibinol, barbiturates, amphetamines and the like; antibiotics, such as gentimycin, and the like; also, the labeled antibodies to these analytes can be used.

Specific pairs of organic species which could be analyzed simultaneously include the following:

1. Carcinoembryonic Antigen (CEA) β-hCG, α fetoprotein, or any other two tumor markers;

2. LH/FSH;

3. Hepatitis B Surface Antigen/Hepatitis B Core Antigen or any other two viral antigens;

4. Thyroxine (T_4)/Thyroid Stimulating Hormone in screening for neonatal hypothyroidism;

5. Thyroxine/Thyroid Binding Globulin (for T3U, i.e. triiodothyronine uptake) in diagnosis and treatment of adult thyroid disease;

6. Angiotensin II/Renin in diagnosing cause for hypertension;

7. Adrenocorticotrophic Hormone (ACTH)/Cortisol in differentiating primary from secondary adrenal disease;

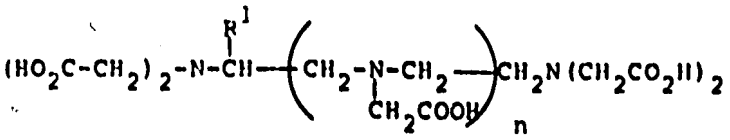
8. Insulin/C-Peptide in the diagnosis and treatment of diabetes;

9. Estriol/Human Placental Lactogen in monitoring pregnancy;

10. Lactate Dehydrogenase (LDH)/Creatine Phosphokinase (CPK) Isoenzymes in diagnosing heart disease; and

11. Serological Screening for Donor Blood for any two viruses or venereal infections simultaneously, such as hepatitis-B Surface Antigen and human T-Cell leukemia virus antigens or antibodies to same.

Chelating agents which can be used include aminopolycarboxylates of the following general formula:



wherein R^1 is phenyl or substituted phenyl wherein the substituents are NO_2, NH_2 and/or SO_3H and the like and n is 0 or 1 such as ethylenediamine tetraacetic acid (EDTA), ethylene dinitrilotetraacetic acid, diethylenetriamine-pentaacetic acid and derivatives thereof such as 1-(p-bromoacetimidobenzyl)-EDTA.

In general any radioisotope of a metal can be employed; however, practical considerations make it convenient to use only those with half lives of a reasonable period of time be. However, it should be understood that even isotopes of a relatively short half life can be employed in this invention.

Preferred isotopes include those of the metals, cobalt, iron, indium, technetium, europium and terbium.

Especially preferred are isotopes of iron and cobalt with cobalt 57 and iron 59 being most preferred.

The labelled organic species may be prepared by reacting the chelating agent with the organic species at a temperature of from 4° to 40°C in a basic solution of a solvent such as sodium bicarbonate (0.1M).

After purification of the organic species, for example by passing the reaction mixture containing it through a molecular sieve, such as sephadex G75, or a polyacrylamide or other polymeric material acting as a molecular sieve, the treatment with the metal isotope at 4°C to 40°C can be carried out in the presence of a buffer from sodium acetate (metal free) or potassium acetate.

To obtain the best products of the invention the buffers should be metal-free if use in the preparation process.

The following examples illustrate the preparation of labelled organic species.

Example 1

PREPARATION OF 57 COBALT LABELLED LH

One mg of lyopholized LH is dry mixed with 3.8 mg of diethylenetriamine pentaacetic anhydride (DTPA). 100 µl of metal-free 0.1 M sodium bicarbonate is added and the reaction vortexed. (metal free buffers are prepared by passing buffer solutions through metal chelating ion exchange resins such as BioRad Chelex 100). After thirty minutes at room temperature, the reaction mixture is passed through a Sephadex G75 column equilibrated and eluted with 0.5 M acetate buffer pH 5.8 (metal free). The LH-DTPA containing fractions are identified by absorbance at 280nm. Peak fractions are pooled and diluted to 100 µg LH/ml with 0.5 M acetate buffer pH 5.8.

100 µl of LH-DTPA (10 ug) are added to 10 µl (500 µCi) of carrier-free 57 cobalt chloride in 0.5 N HCl and reacted for one and one-half hours at room temperature. The reaction mixture is passed through a Sephadex G75 column equilibrated and eluted with phosphate buffered saline (PBS) containing 0.1% bovine albumin. The ^{57}Co-DTPA-LH elutes near the void volume as a single peak. In general >70% of the ^{57}Co is chelated by the LH-DTPA precursor yielding tracers with specific activities ranging from 36 to 43 µCi/µg.

Example 2

PREPARATION OF ^{125}I LABELLED FSH

This procedure describes the process for the preparation of FSH-I^{125} tracer for an iodination size of 5 mCi, which will yield 2 to 2.5 mCi of usable tracer.

37.5 µl of FSH antigen at a concentration of 1 mg/ml in 0.01M PBS is added to a solution of 50 µl of 0.4M phosphate buffer, pH 7.4 and sodium I-125 (5 mCi) and vortexed. The reaction is initiated by the addition of 10 µl of chloramine-T (1 mg/ml 0.1M phosphate buffer, pH 7.2)

to the reaction mixture and vortexed. The reaction is terminated after 25 seconds at room temperature by the addition of 25 µl of sodium metabisulfite (1 mg/ml in 0.1M phosphate buffer, pH 7.4) to the reaction mixture and vortexing.

Immediately after termination, the reaction mixture is transferred to a Sephadex G-75 column (0.5 x 18.0 cm) equilibrated in 0.01 M PBS, 0.1% BSA. The column is eluted with 0.02M PBS/BSA and 0.5 ml fractions (bovine serum albumin (BSA)) are collected. The FSH I-125 elutes between fraction numbers 10 to 20. All fractions which are on the ascending and decending sides of the peak which contain greater than 40% of the activity of the peak tube are pooled. The pooled fractions are diluted with .01M PBS/BSA to a concentration of approximately 100 nCi/ml. The diluted tracer is treated with a 5 ml slurry of Bio-Rad AG-21K resin (rinsed and resuspended in 0.01M PBS, 3% BSA) and stored overnight at 4°C.

By substituting for the tracer organic species and chelating agent in Examples 1 and 2 and by following substantially the procedures described therein, the following radio-labelled tracers can be prepared.

Ex.	Tracer	Organic Species	Chelating Agent
3	^{57}Co	LH	DTPA
4	^{51}Cr	FSH	DTPA
5	^{111}In	TSH	phenyl EDTA
6	^{57}Co	T_4	DPTA
7	^{57}Co	FSH	DPTA
8	^{57}Co	TSH	phenyl EDTA
9	^{57}Co	Ferritin	DTPA
10	^{57}Co	Rabbit anti TSH	DTPA
11	^{57}Co	TSH	DTPA

The following is an example of the types of hormones which can be employed in the assay procedure to be followed and the reagents which are required for the assay.

Organic Species

Luteinizing hormone (LH) and follicle stimulating hormone (FSH) are glycoproteins synthesized and secreted by the basophil (beta) cells of the anterior pituitary in response to gonadotropin releasing hormone (GnRH) produced by the hypothalamus. Both hormones consist of two polypeptide chains designated "alpha" and "beta". The amino acid sequence of the "alpha" subunits is similar for the two hormones as well as TSH and HCG. The "beta" subunits however, are unique and confer immunological specificity, biological specificity and biological activity for the two molecules.

In the female, LH and FSH regulate ovarian changes during the menstrual cycle. FSH promotes maturation of the Graafian follicle and ovum while LH is necessary for the development of a functioning corpus luteum and the production of progesterone. Circulating levels of LH and FSH are controlled by separate negative-feedback mechanisms on the hypothalamus.

In the male, FSH stimulates production of spermatozoa in the seminiferous tubules. Both FSH and LH promote testosterone secretion by the interstitial cells or Leydig tissue of the testes. Testosterone and other steroid hormones control circulating levels of LH and FSH by negative-feedback effects on the hypothalamus.

The measurement of LH and FSH is an important tool for evaluating disorders of the hypothalamic/pituitary/gonadal axis. Hypopituitarism due to pituitary dysfunction in both males and females may result in a hypogonadal state characterised by low levels of LH and FSH (hypogonadotropic hypogonadism). On the other hand,

elevated levels of LH and FSH (hypergonadotropic hypogonadism) may indicate a hypogonadal state caused by primary gonadal failure although LH levels may be normal if androgen secretion is preserved.

In the female, the mid-cycle LH peak is a good indication that ovulation will occur within the next 24 hours. Thus, subfertile couples can be informed of impending ovulation. Such knowledge is also important in timing laparoscopy for oocyte retrieval and subsequent in vitro fertilization.

REAGENTS

LH COBALT 57 TRACER SOLUTION

LH tracer is prepared as described above and diluted to a concentration of approximately 0.02 µCi/ml in 0.01M PBS, 0.1% BSA, 5% normal rabbit serum and 0.1% sodium azide.

FSH ^{125}I TRACER SOLUTION

The FSH tracer solution is diluted in 0.01M PBS, 0.1% BSA, 5% normal rabbit serum, and ion exchange resin strip, and 0.1% sodium azide to a concentration of approximately 0.02 µCi/ml.

LH/FSH ANTISERUM SOLUTION

Each antisera is diluted in 0.01M PBS, 10 mM EDTA, 0.1% BSA, and 0.1% sodium azide at a titer sufficient to bind approximately 30% of the radiolabelled antigens.

LH/FSH PRECIPITATING SOLUTION

Goat anti-rabbit IgG immune serum is diluted in 0.01M PBS, 5% polyethylene glycol and 0.1% sodium azide at a titer sufficient to precipitate 100 microliters of 5% normal rabbit serum.

LH/FSH STANDARDS

Seven concentrations of LH/FSH standards, 0/0, 5/2.5, 10/5, 25/10, 60/25, 120/50, 240/100 mIU/mL are prepared in 0.01M PBS, 0.1% BSA, and 0.1% sodium azide.

LH/FSH CONTROLS

Control samples are prepared in 0.01M PBS, 0.1% BSA and 0.1% sodium azide.

PREPARATION OF REAGENTS

Combine equal volumes of LH ^{57}Co tracer solution and FSH ^{125}I tracer solution. 50 µL of each tracer are required for each assay tube.

SPECIMEN COLLECTION AND PREPARATION

Human serum or plasma samples should be used. If the assay is to be run on the day of specimen collection, store the sample at 4°C until assayed. If the assay is to be run at a later date, store sample frozen at -20°C. Allow the sample to thaw prior to assay; mix thoroughly. Heterogeneity of specimens after thawing has been shown to result in misleading assay values. The sample should be rejected for assay if it is radioactively contaminated from a previous *in vivo* diagnostic procedure. A fresh sample should be drawn after sufficient time has passed for the elimination of the radioactivity from the body.

RADIOIMMUNOASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, sample and assay tubes to room temperature. A standard curve must be performed with each series of unknowns.

Generally, the assay procedure comprises admixing 200 µL of the standards, controls or patient sample with 100 µL of antiserum solution and then vortexing. The mixture is then incubated at 37°C for 60 minutes and thereafter 100 µL of tracer solution are added and the resultant mixture vortexed. After vortexing, the mixture is incubated at room temperature for 60 minutes, 1000 µL of precipitating solution are added and the mixture vortexed. After this vortexing the mixture is incubated at room temperature for 10 minutes and then centrifuged for 15 minutes at 1000 x g. The liquid is then decanted off and the remaining material is counted and the results noted.

More specifically, the assay procedure comprises:-

1. Label 12 x 75 mm assay tubes according to the following outline:

ASSAY TUBE	CONTENTS	
T, T	Total Counts	
1,2	Blank Tubes	
3,4	Standard:	OmIU/Ml
5,6		5mIU/mL LH
		2.5mIU/mL FSH
7,8		10mIL/mL LH
		5mIU/mL FSH
9,10		25mIU/mL LH
		10mIU/mL FSH
11,12		60mIU/mL LH
		25mIU/mL FSH
13,14		120mIU/mL LH
		50mIU/mL FSH
15,16		240mIU/mL LH
		100mIU/mL LH
17,18	C I Control Sample	
19,20	CII Control Sample	
21,100	Patient Sample	

2. Accurately pipette 200 µL of the ZERO STANDARD into the blank tubes and 200 µL of STANDARDS of patient samples into appropriately labelled assay tubes.

3. Pipette 100 µL of ANTISERUM SOLUTION into all tubes except Total Count and Blank tubes and vortex.

4. Incubate at 37°C for 1 hour.

5. Pipette 100 µL of TRACER SOLUTION into all tubes and vortex.

6. Incubate at room temperature (22 ± 3°C) for 1 hour.

7. Shake the PRECIPITATING SOLUTION immediately before use. Pipette 1.0mL into all tubes except total count tubes. Vortex tubes thoroughly.

8. Incubate at room temperature (22 ± 3°C) for 10 minutes.

9. Centrifuge for 15 minutes at 1000 x g.

10. Decant the liquid from each assay tube and blot rims of tubes on absorbent material.

11. Count tubes on gamma counter set respectively for I^{125} CO^{57} for FSH and LH.

PROCEDURAL NOTES

1. Establish a repetitive time pattern for addition of antibody, tracer, and precipitating antibody from the beginning to the end of the assay and for decantation.

2. Be sure all droplets are removed from the rims after decanting.

3. Consistent results occur between assays when a constant room temperature is maintained.

NOTE: The gamma counter must discriminate adequately between ^{125}I and ^{57}Co. Counters that do not permit low crossover between channels or that do not offer adequate stability are unsatisfactory for this assay. Since the energy peaks of Co-57 and I-125 overlap, the windows of the gamma counter must be adjusted to assure <3% crossover. Do not omit this consideration as there is no proportionality in values obtained on an adjusted instrument and the accuracy of the test would be decreased.

$$\text{Using I-125 source: } \frac{\text{CPM from Co-57 channel}}{\text{CPM from I-125 channel}} \times 100 = \text{\% crossover of I-125 into Co-57 channel}$$

$$\text{Using Co-57 source: } \frac{\text{CPM from I-125 channel}}{\text{CPM from Co-57 channel}} \times 100 = \text{\% crossover of Co-57 into I-125 channel}$$

CALCULATION OF RESULTS

1. Average the counts per minute (CPM) for all duplicate tubes. Correct for nonspecific binding by subtracting the average CPM's of tubes 3 and 4 from all other counts.

2. Calculate the % Binding (B/Bo) by dividing the averaged CPM's for the standard and samples by the averaged CPM's of the ZERO STANDARD (tubes 5 and 6) and multiply by 100.

$$\text{Percent Binding (B/Bo)} = \frac{\text{CPM of Standard or Sample}}{\text{CPM of ZERO STANDARD}} \times 100$$

3. Prepare a standard curve on 3-cycle log-logit graph paper by:

   a) Plotting the percent binding (B/Bo) or averaged CPM for each standard concentration on the Y (logit or linear, ordinate) axis and the standard concentration values (mIU/ml LH or FSH) on the X (logarithmic, abscissa) axis.
   b) Draw a straight line through the data points. No attempt should be made to extrapolate the curve beyond the range employed.

4. Read the unknown patient samples from the standard curve (concentration is read off the X axis where patient CPM or % binding intersects the curve).

Typical raw data is shown in Table I.

TABLE I - Typical Data

Tube No.	Contents	LH CPM Bound	LH Average Corrected CPM	LH % B/Bo	LH Patient Value mIU/ml	FSH CPM Bound	FSH Average Corrected CPM	FSH % B/BO	FSH Value mIU/ml
1,2	Totals	90,410 90,576	86,927			67,802 67,808	67,805		
3,4	Assay Blank	3,750 3,548				1,962 1,816			
5,6	O	13,602 13,884	10,094	100		16,494 16,422	14,579	100	
7,8	5/2.5	12,476 12,212	8,691	86.1		14,348 14,160	12,363	84.8	
9,10	10/5	11,574 10,578	7,429	73.6		13,010 11,920	10,570	72.5	
11,12	20/10	9,428 9,626	5,875	58.2		9,638 9,654	7,756	53.2	
13,14	50/25	7,584 7,154	3,725	36.9		5,648 5,635	3,747	25.7	
15,16	100/50	6,018 5,992	2,352	23.3		3,833 3,725	1,895	13.0	
17,18	200/100	4,984 5,034	1,363	13.5		2,801 3,057	1,035	7.2	
19,20	Control I	8,952 9,194	5,424	53.7	25.5	5,248 5,500	3,485	23.9	30.2
21,22	Control	7,678 7,680	4,030	39.9	45.0	9,930 10,484	8,318	57.1	8.7

EXPECTED VALUES (20 - 31)

Normals

	LH mIU/ml	FSH mIU/ml
Female: Follicular phase	0-14	2-10
Mid-cycle peak	10-70	9-18
Luteal phase	0-16	0-9
Post menopause	20-70	20-100
Male:	0-9	2-10

Published LH and FSH ranges may differ because of variations in calibration, method, and/or technique. Each laboratory must confirm its own normal range of a representative sample population.

PERFORMANCE CHARACTERISTICS

Precision is the extent to which a given set of measurements of the same sample agrees with the mean.

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Results of Intra- And Inter-assay Variation:

	Intra-Assay	Inter-Assay	Intra-Assay	Inter-Assay
Pool 1				
X (mIU/ml)	19.4	19.4	6.2	6.2
s (mIU/ml)	1.4	1.9	0.5	0.2
CV (%)	7.1	9.6	7.7	3.3
n	15	15	15	15
m	3	3	3	3
Pool 2				
X (mIU/ml)	41.6	41.6	18.0	18.0
s (mIU/ml)	2.1	2.5	0.9	1.0
CV (%)	5.0	6.1	5.2	5.5
n	15	15	15	15
m	3	3	3	3
Pool 3				
X (mIU/ml)	97.6	97.6	46.3	46.3
s (mIU/ml)	8.4	12.5	2.1	2.7
CV (%)	8.6	12.8	4.6	5.8
n	30	30	30	30
m	6	6	6	6

Sensitivity

Sensitivity is the smallest amount of unlabelled antigen that can be distinguished from no antigen. The sensitivity of the assay is 1.7 mIU/ml for LH and 1.1 mIU/ml for FSH based on 95% B/Bo.

Accuracy

Accuracy is the extent to which a given measurement of a substance agrees with the known value of that substance.

(A) Spike Recovery

Two normal male base pools were spiked with five levels of LH and FSH. Results are shown on the following table:

	LH		FSH	
LH/FSH added (mIU/ml)	X Added Recovered (mIU/ml)	% LH Recovered	X Added Recovered (mIU/ml)	% FSH Recovered
10/5	12.2	122	6.3	127
20/10	21.0	105	11.7	117
40/20	41.7	104	21.6	108
80/40	83.4	104	46.1	115
160/80	130.5	82	65.8	82

(B) Correlation with Other Methods

A patient sample correlation was run against three individual LH radioimmunoassays and three individual FSH radioimmunoassays. A least squares linear regression analysis was then carried out on paired values obtained in the LH and FSH RIA KIT procedure against each of the references. The results are summarized below:

LH: Method A = 0.949 + 1.8
$n = 28$, $r^2 = 0.918$

Method B = 1.314 - 1.6
$n = 28$, $r^2 = 0.959$

Method C* = 0.438 + 5.1
$n = 33$, $r^2 = 0.911$

*Standards for this method are calibrated against 2nd IRP-HMG. All other methods are calibrated against 1st IRP 68/40.

FSH: Method C = 1.045 - 2.8
n = 23, r^2 = 0.893

Method D = 1.686 - 2.1
n = 30, r^2 = 0.977

Method E** = 0.473 - 1.1
n = 30, r^2 = 0.983

**Standards for this method are calibrated against 2nd IRP-HMG. All other methods are calibrated against 1st IRP 69/194.

Specificity

Specificity is the extent of freedom from interference by substances other than the one intended to be measured. The degree of specificity of the antibody for the antigen represents one of the most significant advantages of any radioimmunoassay procedure.

The cross-reactivity of structurally similar hormones at fifty percent binding are given in the following table:

	RELATIVE ACTIVITY*	
COMPOUND	LH ASSAY	FSH ASSAY
LH	1.000	< 0.0035
FSH	0.085	1.000
HCG	0.261	< 0.0028
TSH	< 0.001	< 0.0010

*Relative activity is calculated on a unit/unit basis except for TSH which is calculated on a weight/weight basis.

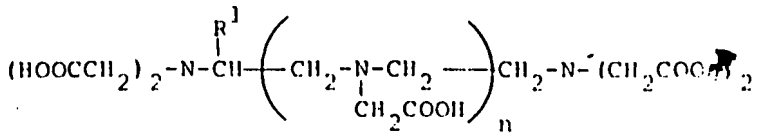
Chelex is a trade mark which may or may not be registered in all of the designated states.

CLAIMS:

1. A coordinated compound of the formula:

metal isotope--chelator--organic species

2. A compound as claimed in claim 1 wherein, for the chelator is used a chelator of the formula:



wherein R^1 is phenyl or substituted phenyl wherein the substituents are NO_2, NH_2 and/or SO_3H and n is 0 or 1, or a derivative thereof.

3. A compound as claimed in claim 2 wherein for the chelator is used ethylenediaminetetraacetic acid, ethylene dinitrilotetraacetic acid, diethylenetriaminepentaacetic acid, or a derivative thereof.

4. A compound as claimed in any of claims 1 to 3 wherein for the metal isotope is used cobalt, iron, iodine, technetium, europium, terbrium or iodine.

5. A compound as claimed in any of claims 1 to 4 wherein for the organic species is used a steroid, a protein, a peptide, a carbohydrate or a drug.

6. A compound as claimed in claim 5 wherein for the organic species is used an estrogen, progesterone, digoxin, cortisol, 17-hydroxyprogesterone, human chorionic gonadotropin, leutinizing hormone, follicle stimulating hormone, thyroid stimulating hormone, alpha-fetoprotein,

trypsin, triiodothyronine, thyroxine, hepatitis associated antigen, carcinoembryonic antigen, adrenocorticotrophic hormone, endorphins, angiotensin, insulin, pneumococal polysaccharides, cocaine, tetrahydrocannibinol, a barbiturate, an amphetamine, gentiamycin, Vitamin B_{12} or folate.

7. A composition useful in a simultaneous assay which comprises two or more coordinated compounds as claimed in any of claims 1 to 6, wherein each metal isotope in each coordinated compound is different.

8. A composition as claimed in claim 7 which comprises two of said coordinated compounds wherein:

(a) for the organic species of one of said coordinated compounds is used carcinoembryonic antigen and for the organic species of the other of said coordinated compounds is used β-hCG;

(b) for the organic species of one of said coordinated compounds is used leutinizing hormone and for the organic species of the other of said coordinated compounds is used thyroid stimulating hormone;

(c) for the organic species of one of said coordinated compounds is used hepatitis B-surface antigen and for the organic species of the other of said coordinated compounds is used hepatitis B-core antigen;

(d) for the organic species of one of said coordinated compounds is used thyroxine and for the organic species of the other of said coordinated compounds is used thyroid stimulating hormone;

(e) for the organic species of one of said coordinated compounds is used thyroxine and for the organic species of the other of said coordinated compounds is used thyroid binding globulin;

(f) for the organic species of one of said coordinated compounds is used angiotensin-II and for the organic species of the other of said coordinated compounds is used renin;

(g) for the organic species of one of said coordinated compounds is used adrenocorticotrophic hormone and for the organic species of the other of said coordinated compounds is used cortisol;

(h) for the organic species of one of said coordinated compounds is used insulin and for the organic species of the other of said coordinated compounds is used C-peptide;

(i) for the organic species of one of said coordinated compounds is used estriol and for the organic species of the other of said coordinated compound is used human placental lactogen;

(j) for the organic species of one of said coordinated compounds is used lactate dehydrogenase and for the organic species of the other of said coordinated compounds is used creatine phosphokinase; or

(k) for the organic species of one of said coordinated compounds is used hepatitis B-surface antigen and for the organic species of the other of said coordinated compounds is used human T-cell leukemia virus.

9. A simultaneous multiple assay wherein one or more coordinated compound as claimed in any of claims 1 to 6 is/are employed.

10. A composition useful in simultaneous assay which comprises one or more coordinated compound as claimed in any of claims 1 to 6 and an analyte labeled with I-125.

11. A composition as claimed in claim 10, wherein the coordinated compound analyte is leutinizing hormone and the analyte labeled with I-125 is follicle stimulating hormone.

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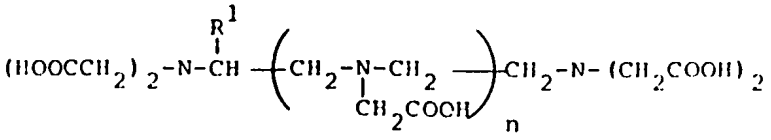
CLAIMS:

1. A method of forming a compound useful in simultaneous multiple assays wherein a metal isotope, chelator and organic species are combined together to form a coordinated compound of the formula:

metal isotope--chelator--organic species

2. A method as claimed in claim 1 wherein for the chelator is used a chelator of the formula:



wherein R^1 is phenyl or substituted phenyl wherein the substituents are NO_2, NH_2 and/or SO_3H and n is 0 or 1, or a derivative thereof.

3. A method as claimed in claim 2 wherein for the chelator is used ethylenediaminetetraacetic acid, ethylene dinitrilotetraacetic acid, diethylenetriaminepentaacetic acid, or a derivative thereof.

4. A method as claimed in any of claims 1 to 3 wherein for the metal isotope is used cobalt, iron, iodine, technetium, europium, terbrium or iodine.

5. A method as claimed in any of claims 1 to 4 wherein for the organic species is used a steroid, a protein, a peptide, a carbohydrate or a drug.

6. A method as claimed in claim 5 wherein for the organic species is used an estrogen, progesterone, digoxin,

cortisol, 17-hydroxyprogesterone, human chorionic gonadotropin, leutinizing hormone, follicle stimulating hormone, thyroid stimulating hormone, alpha-fetoprotein, trypsin, triiodothyronine, thyroxine, hepatitis associated antigen, carcinoembryonic antigen, adrenocorticotrophic hormone, endorphins, angiotensin, insulin, pneumococal polysaccharides, cocaine, tetrahydrocannibinol, a barbiturate, an amphetamine, gentiamycin, Vitamin B_{12} or folate.

7. A composition useful in a simultaneous assay which comprises two or more coordinated compounds of the formula:-

metal isotope--chelator--organic species

wherein each metal isotope in each coordinated compound is different.

8. A composition as claimed in claim 7 wherein the coordinated compounds are prepared by a method as claimed in any of claims 2 to 6.

9. A composition as claimed in claim 7 or claim 8 which comprises two of said coordinated compounds wherein:

(a) for the organic species of one of said coordinated compounds is used carcinoembryonic antigen and for the organic species of the other of said coordinated compounds is used β-hCG;

(b) for the organic species of one of said coordinated compounds is used leutinizing hormone and for the organic species of the other of said coordinated compounds is used thyroid stimulating hormone;

(c) for the organic species of one of said coordinated compounds is used hepatitis B-surface antigen and for the organic species of the other of said coordinated compounds is used hepatitis B-core antigen;

(d) for the organic species of one of said coordinated compounds is used thyroxine and for the organic species of the other of said coordinated compounds is used thyroid stimulating hormone;

(e) for the organic species of one of said coordinated compounds is used thyroxine and for the organic species of the other of said coordinated compounds is used thyroid binding globulin;

(f) for the organic species of one of said coordinated compounds is used angiotensin-II and for the organic species of the other of said coordinated compounds is used renin;

(g) for the organic species of one of said coordinated compounds is used adrenocorticotrophic hormone and for the organic species of the other of said coordinated compounds is used cortisol;

(h) for the organic species of one of said coordinated compounds is used insulin and for the organic species of the other of said coordinated compounds is used C-peptide;

(i) for the organic species of one of said coordinated compounds is used estriol and for the organic species of the other of said coordinated compound is used human placental lactogen;

(j) for the organic species of one of said coordinated compounds is used lactate dehydrogenase and for the organic species of the other of said coordinated compounds is used creatine phosphokinase; or

(k) for the organic species of one of said coordinated compounds is used hepatitis B-surface antigen and for the organic species of the other of said coordinated compounds is used human T-cell leukemia virus.

10. A simultaneous multiple assay wherein one or more coordinated compound of the formula:-

metal isotope--chelator--organic species

is/are employed.

11. A simultaneous multiple assay as claimed in claim 10 wherein the coordinated compound(s) is/are prepared by a method as claimed in any of claims 2 to 6.

12. A composition useful in simultaneous assay which comprises one or more coordinated compound of the formula:-

metal isotope--chelator--organic species

and an analyte labeled with I-125.

13. A composition as claimed in claim 12 wherein the coordinated compound(s) is/are prepared by a method as claimed in any of claims 2 to 6.

14. A composition as claimed in claim 12 of claim 13, wherein the coordinated compound analyte is leutinizing hormone and the analyte labeled with I-125 is follicle stimulating hormone.

European Patent Office

EUROPEAN SEARCH REPORT

0165716
Application number

EP 85 30 3564

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int Cl 4)
X	EP-A-0 038 546 (THE MASSACHUSETTS GENERAL HOSPITAL) * Claims 1-9 *	1-6	G 01 N 33/534 G 01 N 33/60 C 07 C 101/26 G 01 N 33/74 G 01 N 33/94
X	EP-A-0 073 865 (AMERSHAM INTERNATIONAL) * Claims 1-8 *	1-6	
X	US-A-3 994 966 (M.W. SUNDBERG et al.) * Whole document *	1-3	
X	GB-A-2 060 623 (ANALYTICAL RADIATION) * Page 3, lines 30-57; page 6, lines 10-30 *	1-6	
A	EP-A-0 103 558 (WALLAC OY)		TECHNICAL FIELDS SEARCHED (Int Cl 4) G 01 N 33 C 07 C 101
A	EP-A-0 068 875 (EASTMAN KODAK)		

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	01-09-1985	MEYLAERTS H.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
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